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Modeling Single and Repeated Dose Pharmacokinetics of PFOA in Mice

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Perfluorooctanoic acid (PFOA) displays complicated pharmacokinetics in that serum concentrations indicate long half-lives despite which steady state appears to be achieved rapidly. In this study, serum and tissue concentration time-courses were obtained for male and female CD1 mice after single, oral doses of 1 and 10 mg/kg of PFOA. When using one- and two-compartment models, the pharmacokinetics for these two dosages are not consistent with serum time-course data from female CD1 mice administered 60 mg/kg, or with serum concentrations following repeated daily doses of 20 mg/kg PFOA. Some consistency between dose regimens could be achieved using the saturable resorption model of Andersen *et al.* In this model PFOA is cleared from the serum into a filtrate compartment from which it is either excreted or resorbed into the serum by a process presumed transporter mediated with a Michaelis-Menten form. Maximum likelihood estimation found a transport maximum of T_m = 860.9 (1298.3) mg/l/h and half-maximum concentration of K_T = 0.0015 (0.0022) mg/l where the estimated standard errors (in parentheses) indicated large uncertainty. The estimated rate of flow into and out of the filtrate compartment, 0.6830 (1.0131) l/h was too large to be consistent with a biological interpretation. For these model parameters a single dose greater than 40 mg/kg, or a daily dose in excess of 5 mg/kg were necessary to observe nonlinear pharmacokinetics for PFOA in female CD1 mice. These data and modeling analyses more fully characterize PFOA in mice for purposes of estimating internal exposure for use in risk assessment.

Key Words: **perfluorooctanoic acid (PFOA); compartment model; resorption model; pharmacokinetic parameters; statistical analysis; CD1 mice.**

Perfluorooctanoic acid (PFOA) and related compounds are used primarily as surface-active agents in the production of various fluoropolymers and fluoroelastomers (Kudo and Kawashima, 2003). Because of the strength of the carbon-fluorine bond, PFOA is stable to metabolic and environmental degradation (Butenhoff *et al.*, 2004). PFOA is widespread in wildlife and humans—from polar bears living in Greenland (Dietz *et al.*, 2008), to giant pandas in China (Dai *et al.*, 2006), from the general population to occupationally exposed workers (Betts, 2007; Olsen *et al.*, 2007). Average blood levels from the general population in the United States are approximately 4–5 parts per billion (Calafat *et al.*, 2007).

The toxicology of PFOA has been extensively reviewed (Andersen *et al.*, 2008; Lau *et al.*, 2007; Kennedy *et al.*, 2004). PFOA is associated with liver enlargement in rodents and nonhuman primates. Hepatocellular adenomas, Leydig cell tumors, and pancreatic acinar cell tumors occurred in rats (Biegel *et al.*, 2001; Cook *et al.*, 1992). Exposure to a high dose of PFOA (20 mg/kg) for two days late in gestation was sufficient to produce neonatal mortality and birth weight reduction in mice (Wolf *et al.*, 2007). Further investigations showed the daily PFOA treatment with 5 mg/kg and lower doses during gestation was associated with effects (White *et al.*, 2007; Abbott *et al.*, 2007).

PFOA is found in human blood and breast milk from the general population in countries worldwide (Butenhoff *et al.*, 2004). Workers occupationally exposed to fluorochemicals have serum levels of PFOA approximately one order of magnitude higher than those reported in the general population. The PFOA serum elimination half-life in workers was estimated as 3.8 years (Olsen *et al.*, 2007). This is much longer than in laboratory animals, for example, hours for the female rat to days for the male rat to weeks for the monkey (Lau *et al.*, 2007). Gender differences are particularly notable in rats, with limited differences in other animals (Kudo and Kawashima, 2003). The basis for the species and gender differences in elimination of PFOA is still not well understood PFOA is high bound to plasma proteins and this does not appear to differ substantially across species (Kudo and Kawashima, 2003). Differential expression of transporter proteins in the kidney may be one explanation and is clearly a major factor in the sex difference observed in rats (Kudo *et al.*, 2002). Transporter activity has been confirmed in rat, in which organic anion transporters 1 and 3, organic anion transporting polypeptide 1, and

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perhaps others mediate PFOA cross-membrane transport (Kudo *et al.*, 2002; Nakagawa *et al.*, 2008). Recent studies with expressed human and rat organic anion transporters 1 and 2 found similar activity (Nakagawa *et al.*, 2008). In addition, liver distribution in rats is dose-dependent (Kudo *et al.*, 2007) and transporter-dependent (Han *et al.*, 2008), whereas studies in mice have demonstrated that both uptake and efflux transporters in liver are regulated by PFOA (Cheng and Klaassen, 2008; Maher *et al.*, 2008). These kinds of effects presumably underlie the observation of the need to incorporate time-dependent changes in pharmacokinetic modeling for PFOA and PFOS (Harris and Barton, 2008; Tan *et al.*, 2008)

A margin of exposure approach was used in the U.S. EPA's PFOA preliminary risk assessment, which compared measured human blood levels with laboratory animal blood levels associated with toxic effects (U.S. EPA, 2005). The area under the blood concentration-time curve (AUC), concentration at steady state (C_{ss}), or peak concentration (C_{max}) were dose metrics for evaluating effects in this draft assessment. The cross-species pharmacokinetic extrapolation using AUC or C_{ss} (e.g., C_{ss}human/C_{ss}mouse) and a one-compartment pharmacokinetic model is estimated by the ratio of half-lives assuming the volume of distribution is a similar fraction of body weight across species, for example, C_{ss} = dose rate (mg/kg/day)/[volume of distribution (l/kg) × elimination rate constant (1/day)], where half-life equals ln 2/elimination rate constant. To date, the one-compartment model has been used for PFOA pharmacokinetic analysis in rat and monkey (Harada *et al.*, 2005; U.S. EPA, 2005; Washburn *et al.*, 2005). However, the half-life estimated in humans and animals may not be constant and animal half-lives estimated by following blood levels after a single dose may not be comparable with estimates from humans who have had chronic exposure. Monkey and rat data have been interpreted as indicating that the volume of distribution changes with concentration (Trudel *et al.*, 2008; Washburn *et al.*, 2005). Large volumes of distribution do not seem likely, however, because PFOA is known to rapidly achieve quasi-steady-state in blood (Andersen *et al.*, 2006; Lau *et al.*, 2006). Alternatively, monkey data suggests that excretion is concentration dependent (faster elimination rate at higher concentrations), that is, half-lives are not constant (Andersen *et al.*, 2006). These issues increase the difficulty in extrapolating from one species to another. A recently developed biologically motivated pharmacokinetic model of saturable, renal resorption that depends on kinetic factors for transport successfully described the monkey data (Andersen *et al.*, 2006). The difference in apparent elimination rates with increasing dose indicated that capacity-limited, saturable transport processes may be involved in the kinetic behavior of PFOA.

In this study, one- and two-compartment models with first order absorption and clearance were statistically analyzed for PFOA time course data to estimate the pharmacokinetic parameters—volume of distribution, absorption rate, and elimination rate—for female and male CD-1 mice based upon PFOA concentrations following single and repeated doses. The saturable resorption model, which elaborates the description of elimination, also was applied to investigate the kinetic behaviors of PFOA in mice. These analyses characterize models for mice that provide initial estimates of dosimetry that could be applied in risk assessment, though they may also be considered intermediate steps in the development of a more complete physiologically based pharmacokinetic model that would better characterize PFOA tissue distribution, which is not directly addressed by any of these current models.

MATERIALS AND METHODS

PFOA (ammonium salt; >98% pure) was purchased from Fluka Chemical (Steinheim, Switzerland). Nuclear magnetic resonance analysis kindly provided by 3M Company (St Paul, MN) indicated that approximately 98.9% of the chemical was straight-chain and the remaining 1.1% was branched isomers. [1,2-^{13}C]-PFOA was purchased from Perkin-Elmer (Wellesley, MA) and used as an internal standard in the quantitative analysis. For all studies, PFOA dosing solutions were dissolved in deionized water and prepared fresh daily.

Complete data tables are available as an online supplement.

Animal treatment. All animal studies were conducted in accordance with the Institutional Animal Care and Use Committee guidelines established by the U.S. Environmental Protection Agency's Office of Research and Development/National Health and Environmental Effects Research Laboratory. Procedures and facilities were consistent with the recommendations of the 1996 National Research Council's "Guide for the Care and Use of Laboratory Animals," the Animal Welfare Act, and Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Animal facilities were controlled for temperature (20–24°C) and relative humidity (40–60%) and kept under a 12-h light-dark cycle. Mature male and female CD-1 mice (70–80 days of age) were purchased from Charles River Laboratories (Raleigh, NC) and shipped by truck to our facilities, with a transit time of less than one hour. Animals were segregated by sex, housed in polypropylene cages (three per cage), and provided pellet chow (LabDiet 5001, PMI Nutrition International, St. Louis, MO) and tap water *ad libitum*. Mice were allowed several days for acclimation and randomly assigned to treatment groups. Several studies were undertaken involving single or repeated dosing. Two studies with very similar designs were carried out in which mice were given a single oral gavage treatment of either 1 mg/kg or 10 mg/kg PFOA. In the first study (PK1), three males and three females from each dose group were sacrificed by decapitation at the following time intervals: 4, 8, or 12 h, and 1, 3, 6, 9, 13, 20, 27, 34, 42, or 48 days. Trunk blood was collected for serum preparation and stored at −20°C; liver and kidney were dissected, flash-frozen on dry-ice and stored at −80°C until being processed for analysis. For the second study (PK2), the evaluation time points were extended to include 55, 62, 70, and 80 days. Serum, liver and kidneys were collected and stored as described previously. Based upon initial modeling efforts, a study at a higher dose was carried out in which female mice were given 60 mg/kg PFOA (6 mg/ml dosing solution, 10 ml/kg dosing volume) and three mice were sacrificed at each of the following time intervals: 2, 4, 6, 8, 12, 24, 36 h, or 2, 4, 6, 8, 11, 14, 21 days, 28 days. Only serum was analyzed for these animals. Finally, a repeated dose study was carried out in which five animals received 20 mg/kg/day for 17 days and serum was obtained 24 h after the final dose as previously described (Lau *et al.*, 2006).

PFOA determination. Serum samples were thawed and mixed well by vortexing; an aliquot (25–100 μl) was removed for analysis. The volume of serum assayed was varied to optimize detection of PFOA because levels were very high at early time points and very low at the latest. Liver and kidney were thawed, weighed and homogenized (polytron) in 5 volumes of deionized, distilled water. Analysis of PFOA in serum and tissues was performed using a modification of a method originally developed by Hansen *et al*. (2001). Briefly, 25–100 μl of serum or 25 μl of tissue homogenate was combined with 1 ml of 0.5M tetrabutylammonium hydrogen sulfate (pH 10) and 2 ml of 0.25M sodium carbonate and then vortexed for 20 min in a 15 ml of polypropylene tube. Three hundred microliters of this mixture was then transferred to a fresh 15 ml of polypropylene tube and 25 μl of a 1 ng/μl solution of 13C-PFOA was added as an internal standard. Five milliliters of methyl tert-butyl ether (MTBE) was then added and vortexed again for 20 min. The sample was centrifuged at 2000 × g for 3 min to separate the aqueous and organic phases, and 1 ml of the MTBE layer was transferred to a 5-ml polypropylene tube where it was evaporated to dryness at 45°C under a gentle stream of dry nitrogen. The residue was then solubilized in 400 μl of a 2mM ammonium acetate/acetonitrile (1:1 by vol) solution and transferred to a polypropylene autosampler vial. No pH adjustments were made for this solution. Extracts were analyzed using an Agilent 1100 high-performance liquid chromatograph (Agilent Technology, Palo Alto, CA) coupled with an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) (LC/MS/MS). Ten microliters of the extract was injected onto a Luna C18(2) 3 × 50 mm, 5-μm column (Phenomenex, Torrance, CA) using an isocratic mobile phase consisting of 30% 2mM ammonium acetate solution and 70% acetonitrile at a flow rate of 200 μl/min. PFOA and 13C2-PFOA were monitored using parent and daughter ion transitions of 413 → 369 and 415 → 370, respectively. Peak integrations and areas were determined using Analyst software (Applied Biosystems Version 1.4.2, Foster City, CA). For each analytical batch, matrix-matched calibration curves were prepared as described above using mouse serum spiked with varying levels of PFOA. For quality control, check standards were prepared by spiking large volumes of mouse serum at several arbitrary levels. These check standards were stored frozen and aliquots analyzed with each analytical set. Different preparations of standards were used for each experimental study; the concentration of each newly prepared standard was compared with the previous batch to ensure consistency. In addition, control mouse serum samples were fortified at two or three levels in duplicate with known quantities of PFOA during the preparation of each analytical set. Duplicate fortified and several check standards were run in each analytical batch to assess precision and accuracy. The limit of quantification (LOQ) was set as the lowest calibration point on the standard curve. Analytical batches were considered to be acceptable if: matrix and reagent blanks had no significant PFOA peaks approaching the LOQ, the standard curve had a correlation coefficient > 0.98, and all standard curve points, fortified, and check samples were within 70–130% of the theoretical and previously determined values, respectively.

One- and two-compartment pharmacokinetic analysis of 1 and 10 mg/kg data. The PFOA single oral dose time course data at the two lower doses included three tissues (blood sera, liver and kidney), two genders (female and male mice), and two doses (1 and 10 mg/kg) collected in two experimental blocks (PK1 and PK2). Thus, there are 24 data sets in all. We estimated parameters using R (version 2.4.1, R Development Core Team, 2007). One- and two-compartment models were fit to blood sera, liver, and kidney time-course data for each gender, dose, and block.

The one- and two-compartment models with first order absorption and first order elimination can be described as:

$$C(t) = \frac{k_a D}{(k_a - k_e)/V_d}\left(e^{-k_e t} - e^{-k_a t}\right)$$

One-compartment model, where D, dose; V_d, volume of distribution; k_a, adsorption rate constant; k_e, elimination rate constant.

$$C(t) = \frac{k_a D}{V_1}\left[\left(\frac{k_{21} - \alpha}{(k_a - \alpha)(\beta - \alpha)}\right)e^{-\alpha t} + \left(\frac{k_{21} - \beta}{(k_a - \beta)(\alpha - \beta)}\right)e^{-\beta t} - \left(\frac{k_{21} - k_a}{(\alpha - k_a)(k_a - \beta)}\right)e^{-k_a t}\right]$$

Two-compartment model, central compartment, where D and k_a are as above and V_1, volume of central compartment; k_{12}, rate constant for transfer from compartment 1 to compartment 2; k_{21}, rate constant for transfer from compartment 2 to compartment 1; α, agglomerate rate constant representing net loss from the central compartment during the distribution phase; β, agglomerate rate constant representing net loss from the central compartment after the distributional phase is complete.

The models were fit by using generalized nonlinear least square (gnls) using the *R* function gnls in package nlme (Pinheiro *et al*., 2007), to estimate the parameters. The likelihood ratio test was applied to compare the one- and two-compartment models to determine which model better described the data.

Initially, one-compartment models were fit with a separate parameter value for each gender, dose-level, and experimental block in each tissue. Conditional *F*-tests (Pinheiro and Bates, 2000) on linear combinations of the dose-block-gender specific parameters were then used to determine the extent to which each parameter could be simplified. An orthogonal series of contrasts, analogous to those in multi-factorial analysis of variance, was developed, so that interaction terms were first tested (in order of decreasing complexity) followed by main effects terms. That is, we first tested for a given parameter type (e.g., volume of distribution) whether there was a significant three-way interaction (dose × block × gender), which was followed by (if the three-way interaction was not significant) dose × block, dose × gender, block × gender, and then gender, block and dose. Using the results of these tests, a new statistical model was constructed by collapsing over the effects that were not significant. For example, if only gender effects remained significant for volume of distribution, a new model would be constructed in which volume of distribution was allowed to vary among genders, but not across blocks or dose levels. When block was found to be significant, it was incorporated as a random effect in a nonlinear mixed-effects model, fit using the function nlme.

One- and two-compartment pharmacokinetic analysis of 60 mg/kg data. Subsequent to the analysis of 1 and 10 mg/kg data, an oral time course in serum of female mice exposed to 60 mg/kg was collected based upon preliminary model predictions that the time course should be biphasic. This data was also evaluated for one- and two-compartment model fits.

The data differ from that collected for 1 and 10 mg/kg doses in having replicate values for about half the measurements, so a hierarchical statistical model was fit to the data, with V_d (in the one-compartment model) and V_1 (in the two-compartment model) varying among subjects. k_e and k_a were found to not be statistically identifiable as individually varying parameters. In this model, the distribution of the log of the compartment volume is assumed to be Gaussian, and the population mean and variance are additional parameters to be estimated. Estimation was via the method of Lindstrom and Bates (1990), as implemented in the package nlme (Pinheiro *et al*., 2007) for R (R Development Core Team, 2007).

Saturable resorption model analysis. Our saturable resorption model was adapted with minor modifications from Andersen *et al.* (2006), and implemented using Matlab (version R2007a, The Mathworks, Natick, MA) (see Appendix) to simulate and predict the single and repeated oral dose data for blood sera in female mice. Solutions were obtained using a stiff solver (ode23s) that implemented the modified Rosenbrock (2,3) pair approach (Shampine and Reichelt, 1997). All the simulations were run on a computer equipped with 3-GHz Dual Core Pentium 4 processor and the Windows XP operating system.

The salient feature of the Andersen *et al.* (2006) model is that the free concentration of PFOA in the central compartment (given by free* C_1) is cleared to a filtrate compartment where it is either excreted or resorbed via a saturable process with a Michaelis-Menten form. We examined the original three compartment (two body, one filtrate) model with eight model parameters

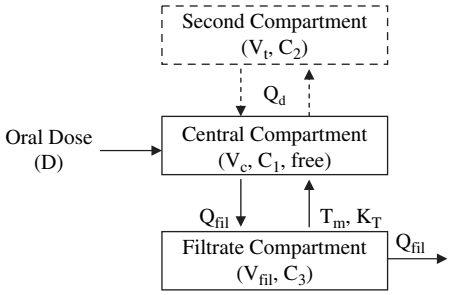

FIG. 1. A schematic for the renal saturable resorption pharmacokinetic model.

as well as a simplified, two-compartment (one body, one filtrate) model with six parameters (Fig. 1). Oral uptake was assumed to be first order with the same rate we determined for the one-compartment model.

We determined the model parameters for female mice via Maximum Likelihood Estimation. Values of the likelihood function were calculated for eight sets of observations in sera: the two blocks each of 1 mg/kg and 10 mg/kg single doses, the 60 mg/kg single dose, the 17-day repeated 20 mg/kg/day dose observations, and the repeated dose data from Lau *et al.* (2006) for 7 and 17 days also at 20 mg/kg/day. We allowed the coefficient of variation to be different for each of these eight sets of observations so that our likelihood function depended upon either six or eight model parameters and eight variance parameters. The contribution to the likelihood of for each animal was calculated—for a few animals where replicate measures had been performed, the results were averaged. We used a Nelder-Mead optimizer (Lagarias *et al.*, 1998) to find the combination of parameters that maximized the likelihood. We numerically approximated the second derivative (D'Errico, 2007) of the likelihood function at the optimized parameter values to obtain the standard error for each parameter estimate.

RESULTS

Experimental Data

Serum, and in some cases tissue concentrations, of PFOA in mice were obtained in several studies including time course data following a single dose at three different dosages and single time points following two durations of repeated dosing (Table 1).

The blood sera, liver and kidney concentration time-courses after single oral doses of 1 and 10 mg/kg are plotted in Figure 2 for the male and female mice. Male and female mice fairly rapidly absorbed PFOA, as judged by the time of maximum observed concentration (4 or 8 h). The liver concentrations were often higher than those in sera, whereas both were substantially higher than the kidney concentrations. The data from sera, liver, and kidney and plots for all the male and female mice dosed with 1 and 10 mg/kg are presented in the Supplemental Materials.

For single doses of 1 and 10 mg/kg the pharmacokinetics are essentially linear, as illustrated in Figure 3 in which the female serum time courses collapse, when scaled by dose, onto approximately the same line on a semilogarithmic plot. The pharmacokinetics were quite different at the highest dose, 60 mg/kg, appearing roughly bi-exponential with low concentrations, as a fraction of total dose, achieved more rapidly.

Statistical Analysis of Compartmental Pharmacokinetic Models

Pharmacokinetic data are routinely analyzed using classical one and two-compartmental models and serum concentration data (Table 2). To compare clearance and apparent volume of distribution, as reflected by liver and kidney concentrations, data for each of these tissues were also fitted using the compartmental models. For the 1 and 10 mg/kg data, all kinetic parameters were identified and estimated in the one-compartment model. With the two-compartment model, it was possible to estimate parameters in only six of the twenty-four 1 and 10 mg/kg data sets for blood sera, liver and kidney, due to failures of convergence. These failures are likely to be related to the inability to uniquely estimate some of the parameters. We compared the one- and two-compartment models for estimating the available corresponding identified parameters using the likelihood ratio test and found that none of the results were significant ($p > 0.05$), that is, adding a second compartment did not significantly improve the ability of the model to account for the data. Thus, we at first focused on the one-compartment model for further parameter estimation studies with the 1 and 10 mg/kg data.

V_d and k_e differed significantly between males and females for all three tissues, although the differences generally are not large (Table 2). V_d differed between doses in kidney. V_d also varied significantly between the two blocks in sera, which was therefore included in the estimation of experimental error (all $p < 0.05$). The absorption rate constant, k_a, was marginally estimable with these data, so it was estimated as a single value across all data sets for each tissue.

TABLE 1
Pharmacokinetic Studies of PFOA in Mice

	Dose	Sex	Tissues	Sampling
Single dose (PK 1)	1, 10 mg/kg	Male, female	Serum, liver, kidney	Time course
Single dose (PK 2)	1, 10 mg/kg	Male, female	Serum, liver, kidney	Time course
Single dose	60 mg/kg	Female	Serum	Time course
Repeated dose	20 mg/kg, 17 days	Female	Serum	24 h after final dose
Repeated dose; Lau *et al.* (2006)	20 mg/kg, 7 and 17 days	Female	Serum	24 h after final dose

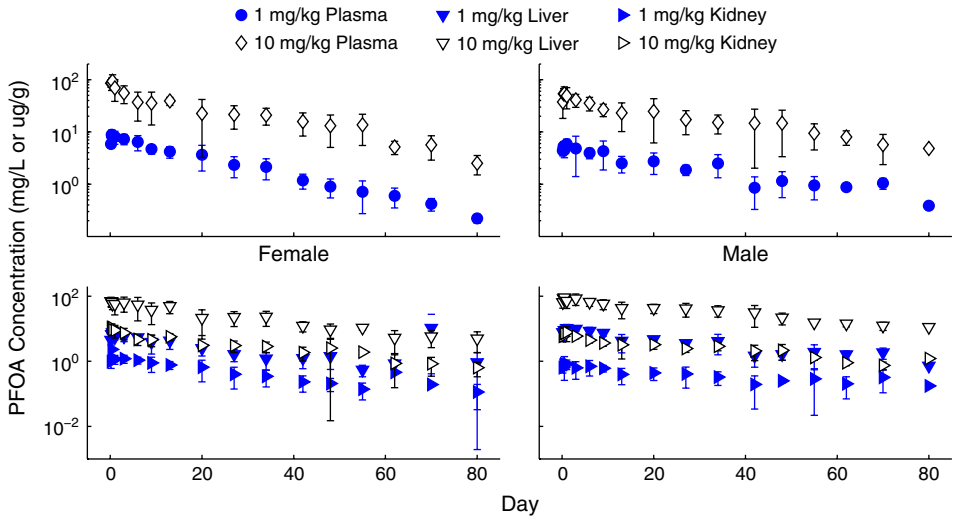

FIG. 2. Experimental data plots for different doses, genders, and blocks.

The mean and 95% confidence intervals for each parameter are shown in Table 2. Because the initial sampling time point 4 h, is too late to capture the absorption processes, the 95% confidence intervals for k_a for blood sera and liver are quite wide. We cannot identify k_a for kidney from our data. To solve this problem, we first explored the sensitivities of estimates of V_d and k_e to values of k_a by estimating V_d and k_e while fixing k_a at 0.3, 0.5, 1, and 1.5/h. The estimates and 95% confidence intervals were insensitive to the particular value chosen for k_a, so k_a for kidney was set to the mean (0.527/h) of k_a values in blood sera and liver. As shown in Table 2, female mice have lower values of V_d in blood sera and kidney, but higher values in liver, than male mice. In all the three tissues, k_e values are modestly higher in female mice than in male mice.

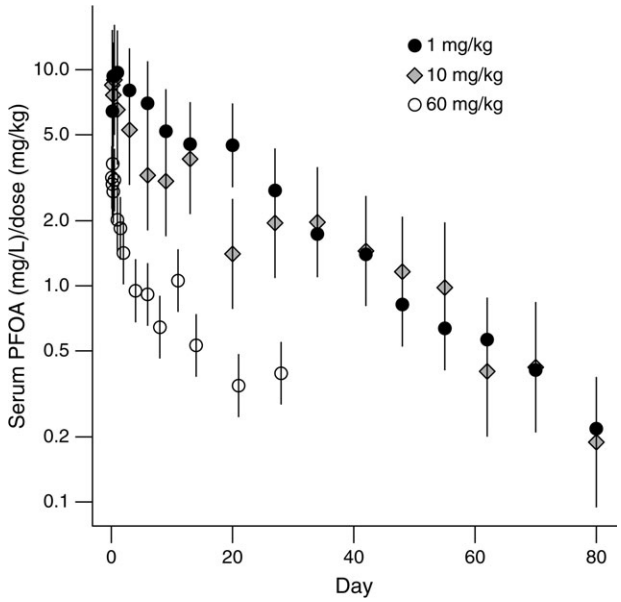

FIG. 3. Serum concentrations scaled by dose for females administered single doses of 1, 10, and 60 mg/kg. Points are means, error bars are 95% confidence intervals for the means. 1 and 10 mg/kg dose groups are largely superimposed and linear in time on this semi-log suggesting linear first-order kinetics at these doses. The 60 mg/kg group has a substantially different shape and time course.

The one-compartment model was successful in describing the 1 and 10 mg/kg single dose serum data sets with estimated values of V_d (= 0.135 l/kg) and k_e (= 0.00185/h) for female mice. These same two parameter values fail to predict serum concentrations for both the higher, 60 mg/kg single dose and repeated dose (20 mg/kg) blood sera data.

The serum concentrations resulting from doses of 10 and 60 mg/kg, converge after roughly a day, indicating that linear pharmacokinetic models—including both one and two-compartment models—cannot reproduce the observed pharmacokinetics. Though the 60 mg/kg single dose data was well-described by a two-compartment analysis (Table 3), as is shown in Figures 4a and 4b, predictions made using the two-compartment parameter estimates from the 60 mg/kg data were not consistent with the 1 and 10 mg/kg blood sera data. Jointly analyzing all the available data to optimize the two-compartment model, also shown in Figure 4, resulted in predictions that did not reproduce any of the dose regimens.

After 7 and 17 days of dosing female CD1 mice with 20 mg/kg PFOA, Lau *et al.* (2006) found that the measured serum levels were approximately equal (176 ± 56 vs. 172 ±34 mg/l, respectively). We use recalculated values based on the original, individual mouse data that are somewhat different from what was reported by Lau *et al.* (2006). Additional 17 day, 20 mg/kg repeated dose experiments were performed for five female CD1 mice and a serum PFOA concentration of 130 ± 23 mg/l was measured.

The one-compartment model only fit the repeated dose data if the elimination rate k_e was increased from 0.00185/h to 0.0255/h, that is, the half-life of PFOA in blood sera decreased from 15 to 1.2 days. This results in the contradiction that different kinetics were observed when using the one-compartment

TABLE 2
One compartment model parameters for 1 and 10 mg/kg doses of PFOA

			Female (95% confidence interval)	Male (95% confidence interval)
Blood Sera	$V_{d\ (L/kg)}$		0.135 (0.102-0.179)	0.226 (0.202-0.253)
	$k_{a\ (1/h)}$		0.537 (0.300-0.960)	
	$k_{e\ (1/h)}$		0.00185 (0.00175-0.00196)	0.00133 (0.00120-0.00148)
	$>t_{½\ (day)}$		15.6 (14.7 – 16.5)	21.7 (19.5 – 24.1)
Liver	$V_{d\ (L/kg)}$		0.161 (0.148-0.176)	0.120 (0.111-0.129)
	$k_{a\ (1/h)}$		0.517 (0.303-0.881)	
	$k_{e\ (1/h)}$		0.00161 (0.00143-0.00181)	0.00129 (0.00115-0.00145)
Kidney	$V_{d(L/kg)}$	1 mg/Kg	0.822 (0.745-0.908)	1.280 (1.145-1.432)
		10 mg/Kg	1.092 (1.004-1.188)	1.700 (1.520-1.902)
	$k_{a\ (1/h)}$		0.527	
	$k_{e\ (1/h)}$		0.00151 (0.00138-0.00166)	0.00113 (0.000992-0.00128)

model to predict the single and repeated dose experimental data, though whether this was a consequence of dose-dependent differences or the system not being stationary and exhibiting kinetic changes with exposure could not be differentiated based upon these data alone.

The two-compartment model predictions for repeated doses, shown in Figure 4c, did not show the rapid approach to steady state that had been observed following 7- and 17-day repeated dose observations. Neither parameters optimized using the 60 mg/kg single dose data alone nor those obtained from optimizations performed with all the single and repeated dose data provided a good description of repeated dose pharmacokinetics.

The saturable resorption model (Andersen *et al*., 2006), on the other hand, can produce results that are consistent not only with the 1, 10, and 60 mg/kg single dose data (Fig. 5a), but also consistent with repeated dose data (Fig. 5b). We performed a maximum likelihood optimization using both the single and repeated dose data sets (Table 4). Simulation results indicated that with repeated doses the PFOA serum concentrations reached pseudo-steady state very rapidly (two days after the first dosing), and the minimum and maximum serum levels were approximately 125 and 180 mg/l. The new 17-day repeated dose concentrations were well predicted, whereas the 7- and 17-day data from Lau *et al*. (2006) were underpredicted by our parameter estimates, although within the 95% confidence limits. Confidence limits are calculated using 200 random draws using the estimated covariance matrix for the likelihood function. When we analyzed the single dose data sets alone, we found that the repeated dose concentrations observed by Lau *et al*. (2006) were greatly underpredicted.

TABLE 3
Two-Compartment Model Parameters for 60 mg/kg Doses of PFOA in Female Mouse Serum

Parameter	Value (95% confidence interval)
Absorption rate (k_a)	1.05 (0.27–4.01) /h
Volume of distribution in the central compartment (V_c)	0.27 (0.22–0.34) l
Rate constant for transfer from compartment 2 to compartment 1 (k_{21})	0.012 (0.0065–0.023) /h
α	0.037 (0.020–0.067) /h
β	0.0017 (0.0011–0.0026) /h

As indicated by Figure 6, the saturable resorption model allows linear behavior at low doses before switching to much more rapid clearance at high doses. This is evident in the predicted filtrate concentration time-course shown in Figure 6—once the transport from the filtrate to the primary compartment saturates, the concentration in the filtrate compartment is predicted to spike so PFOA is rapidly eliminated.

As a measure of model performance, we calculated the Akaike Information Criterion (AIC) (Akaike, 1974) for the resorption models with one and two body compartments. We found that with one body compartment the AIC was 314.37, whereas with two body compartments the AIC was 293.58. The lower AIC for the two body compartment model indicated that the additional model parameters necessary to describe the second body compartment were supported by better fitting of the data.

Andersen *et al*. (2006) found that the saturable model was not strongly sensitive to the value of the filtrate compartment and used an arbitrary value of 0.1 l. Using the AIC, we similarly found that the marginal increase in likelihood introduced by including the filtrate volume is not offset by the uncertainty of an additional parameter. Additionally, we found that the available data did not support estimating the free fraction of PFOA. For both the filtrate volume and free fraction we assumed the values used by Andersen *et al*. (2006). The optimized parameter values are reported in Table 4.

The estimated parameter uncertainties for Q_{fil}, T_m, and K_T are all quite large; the estimated standard errors are larger than their mean values. This combination of parameters seems to be weakly identifiable given the available data. Assuming a base value for overall cardiac output of 16.5 l/h and scaling by a factor of 0.025^(3/4) then the total mouse cardiac output should be 1.04 l/h. In this case, the optimized value of Q_{fil}

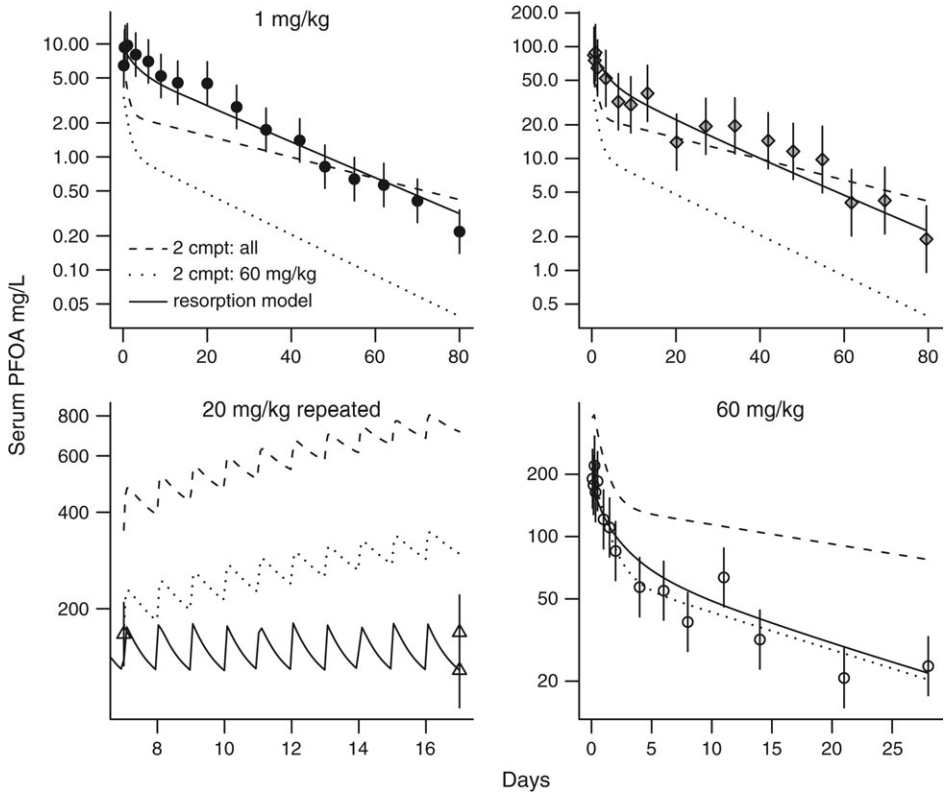

FIG. 4. Comparing predictions for the two-compartment model when fit to all the available data (dashed line) with a fit to just the 60 mg/kg data (dotted line). Neither model does a good job of describing all of the data, whereas the saturable resorption model (solid line) is more consistent between doses.

(0.6830 l/h) is roughly two thirds of the total output. For comparison, in monkeys Andersen *et al*. (2006) assumed that Q_{fil} was 10% of cardiac output to the kidney, corresponding to $Q_{\text{fil}} = 0.0943$ l/h. For a standard body weight of 0.025 kg, glomerular filtration for female C57BL/6J mice is much smaller, 0.00945 l/h (Qi *et al*., 2004), whereas in adult male CD1 mice the urinary flow rate is even smaller—0.000076 l/h (Luippold *et al*., 2002). Thus, our optimized value for Q_{fil} does not appear to have a direct physiologic analog.

Using our calibrated saturable resorption model, we investigated what doses of PFOA are predicted to show the two different kinetic behaviors. For selected dose levels, we predicted changes in kinetics from low dose to high dose (Fig. 7). Only one phase was predicted at low doses (< 40 mg/kg),

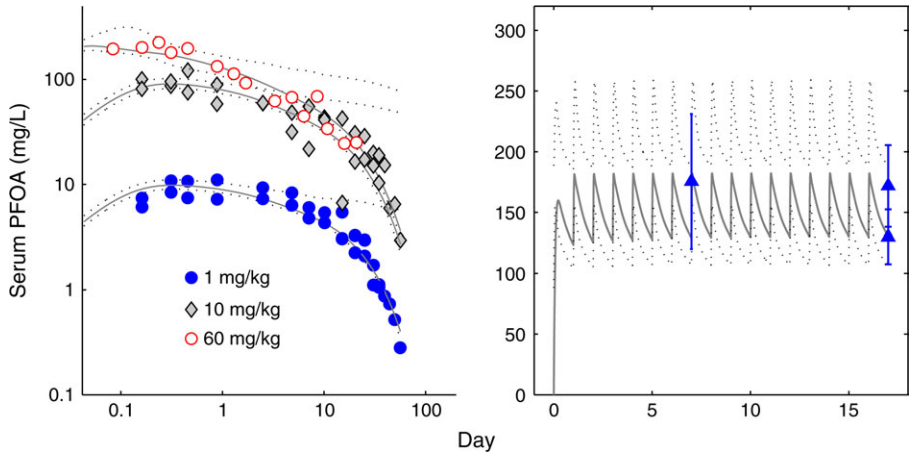

FIG. 5. Predictions and quantiles for the saturable resorption model when optimized using all available data. The predictions for the maximum likelihood estimated parameters are indicated by a solid line, with open squares indicating where model predictions should be compared with observations for the repeated dose data. Dashed lines indicate the 95% upper and lower quantiles using the estimated parameter uncertainty. The Lau *et al*. (2006) 7- and 17-day observations as well as our new 17-day observations are indicated by solid triangles.

TABLE 4
Assumed and Optimized PFOA Resorption Model Parameters

Parameter	Value (SE)	Source
Body weight (BW)	25 g	Assumed standard value
Cardiac output	16.5 l/h for mice	Barbee *et al.* (1992)
Absorption rate (k_a)	0.537 /h	Estimated from single dose data using one-compartment model
Volume of distribution of the central compartment (V_c)	0.0027 (0.0002) l	Optimized
Volume of renal filtrate (V_{fil})	0.01 l	Assumed, Andersen *et al.* (2006)
Renal blood filtrate rate (Q_{fil})	0.6830 (1.0131) l/h	Optimized
Volume of distribution of second body compartment (V_t)	0.0545 (0.0151) l	Optimized
Intercompartmental clearance (Q_d)	0.00059 (0.00037) l/h	Optimized
Transport maximum (T_m)	860.9 (1298.3) mg/l/h	Optimized
Transport affinity constant (K_T)	0.0015 (0.0022) mg/l	Optimized
Proportion of PFOA free in serum (free)	0.02	Assumed, Andersen *et al.* (2006)

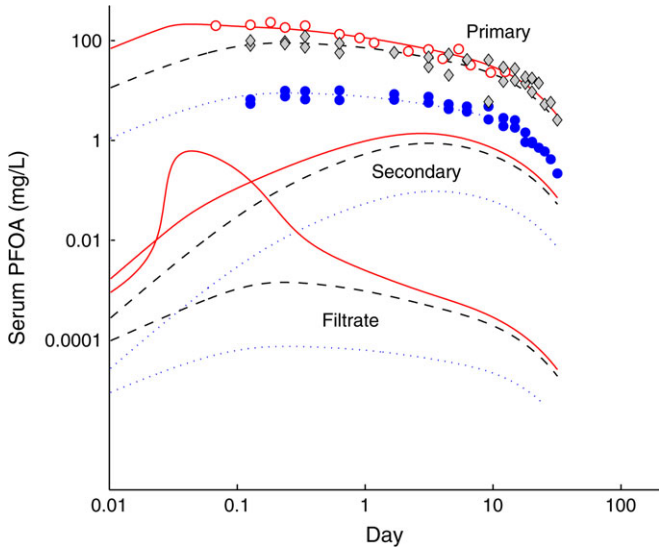

FIG. 6. Saturable resorption model predictions using parameters obtained with a maximum likelihood estimate show that the concentration in the filtrate compartment (dashed) spikes early on allowing the concentration in the primary compartment (solid) to rapidly converge for doses of 10 and 60 mg/kg.

whereas two phases occurred at the high doses (> 40 mg/kg) with a fast initial elimination rate giving way to a much slower rate after roughly one day. Simulations using an earlier version of the model were the basis for selecting the 60 mg/kg dose, which did demonstrate biphasic behavior predicted by the saturable resorption model but not previously observed in the serum time course data with lower doses. For repeated doses, daily doses of 0.01, 0.1, and 1 mg/kg saturated after about two weeks, whereas for 5 mg/kg the serum concentration quickly saturated. Within a day of daily doses of 50 and 500 mg/kg, serum concentration saturated at the same concentration as with 5 mg/kg.

Normalized sensitivity coefficients, defined as (change of output/output)/(change of input/input), were used to test the parameter sensitivity at different days after a single dose of 20 mg/kg (Fig. 8). After 1 day, the most sensitive parameters were Q_{fil}, T_m, and K_T, that is, the kinetics of the resorption process, because they dictate clearance, are the most important for predicting long-term concentrations.

DISCUSSION

For 1 and 10 mg/kg single doses, kinetic parameters differ significantly between genders but the magnitude of the differences are small indicating PFOA pharmacokinetic behaviors are similar in female and male mice in contrast to rats. The values of the parameter k_a were not well estimated, and the 95% confidence intervals were wide. This is because the PFOA absorption in mice was fairly rapid, and the absorption was almost finished before the initial sampling time point (4 h). Due to the uncertain estimation of k_a values, we used only one k_a for female and male mice for each tissue.

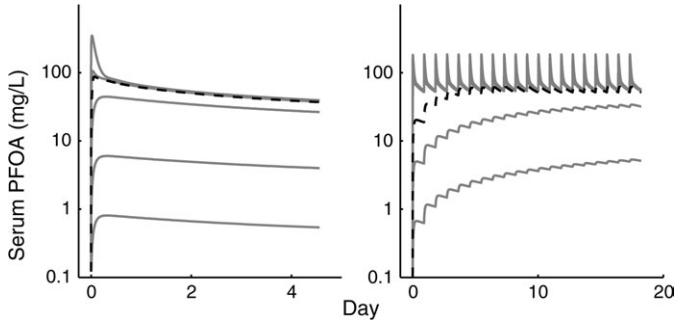

FIG. 7. Delineation of predictions for the PFOA concentration (mg/l) in the central compartment. For the single dose (top) solid lines depict doses of 0.1, 1, 10, 100, and 1000 mg/kg. The dashed line indicates a dose of 40 mg/kg which is roughly where the onset of nonlinearity occurs. For the repeated dose (bottom) solid lines depict repeated daily doses of 0.001, 0.1, 1, 50, and 500 mg/kg. The dashed line indicates a daily dose of 5 mg/kg.

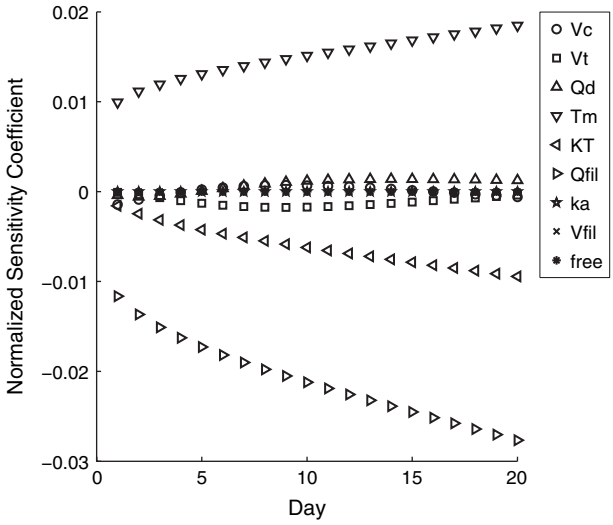

FIG. 8. Analysis of the parameter sensitivity by increasing each parameter in turn by 1% and comparing predicted concentrations for a 20 mg/kg single dose with those for the optimized/assumed value. Note that plot points for several parameters are on top of each other near zero.

The one-compartment model is successful in describing the 1 and 10 mg/kg single dose data sets with the estimated values of V_d, k_a, and k_e, but fails in predicting the higher, 60 mg/kg single dose and the repeated dose data. Similarly, although the 60 mg/kg data can be described by a two-compartment model, for the optimized parameters that model overestimates the 1 and 10 mg/kg single dose data. Neither model predicts the repeated dose observations without changing some model parameters drastically from the single dose case.

The saturable resorption model of Andersen *et al*. (2006) reconciles the lower two doses with the high single dose by allowing the clearance to change for different exposure levels in place of the first order or proportional clearance in the previous 1 and 2 compartment models. At low dose or the early period of repeated dose for our data, the PFOA concentration in the filtrate compartment is low and is proportional to dose, which has a low net urine elimination rate, whereas at high dose (including pseudosteady state of repeated dose for our data), the PFOA concentration in the filtrate compartment is high and resorption is saturated, which results in a high net urine elimination rate.

The saturable resorption model does not, however, completely reconcile the single dose data with the repeated dose concentrations. Though parameters can be estimated such that most data is with the 95% confidence intervals, the concentrations observed after repeated doses by Lau *et al*. (2006) seem to be systematically higher than predicted. This may reflect experimental variability in light of the repeated dose data reported here, which was lower than that previously measured.

The modeling analyses presented here can be used to estimate initial internal dose metrics for toxicity studies carried out in adult mouse. Characterization of the uncertainties in the parameter estimates permits some description of the uncertainties in predicted dose metrics. However, none of these models describe tissue dosimetry, which would require a physiologically based pharmacokinetic model structure. The recent demonstrations that PFOA exposures in mice alter transporter expression in at least the liver (Cheng and Klaassen, 2008; Maher *et al*., 2008), raise further questions about the time course and dose-response for these changes and how they affect serum, liver, or other tissue concentrations. Additional experimental work would also benefit from quantifying the fecal and urinary elimination from mice because current analyses assume nearly complete absorption and do not distinguish elimination routes. Models that are more physiologically based would potentially need to explicitly address these issues.

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APPENDIX

```
function [dCdt]=pfoa_ode_new2compab(t,C,P)
% From one-compartment analysis of 1 and 10 mg/kg data:
ka=0.537;
% From Andersen et al. (2006):
Vfil = 0.01;                                    %[Vfil] = L
free = 0.02;                                    %[free] = 1
% Parse the parameter vector P
Vc = P(1);                                      % [Vc] = L
Vt = P(2);                                      % [Vt] = L
kd = P(3);                                      %[kd] = 1/h
Qd = kd*Vc;                                     %[Qd] = L/h
Tm = P(4);                                      %[Tm] = mg/L/h
KT = P(5);                                      %[KT] = mg/L
kfil = P(6);                                    %[kfil] = 1/h
Qfil = kfil*Vc;                                 %[Qfil] = L/h
% Note that gut compartment has different units:
dCdt=zeros(4,1);
dCdt(1) = ka/Vc*C(4)-Qd/Vc*free*C(1)+Qd/        %[C(1)] = mg/L
  Vc*C(2)-Qfil/Vc*C(1)*free+
  Tm*C(3)/(KT+C(3));
dCdt(2) = 1/Vt*(free*Qd*C(1) - Qd*C(2));        %[C(2)] = mg/L
dCdt(3) = 1/Vfil*(Qfil*C(1)*free-Vc*Tm*C(3)/    %[C(3)] = mg/L
  (KT+C(3))-Qfil*C(3));
dCdt(4) = -ka*C(4);                             %[C(3)] = mg
```

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